

An apparent mutualism between Afrotropical ant species sharing the same nest

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Abstract

Social insects have a highly developed nestmate and species recognition system that is quite effective at keeping out unwanted intruders. Rare cases of “parabiosis,” however, are known in some ants where two species apparently live peacefully alongside each other within the same nest. Here, we report on such an association between the tiny Afrotropical ant *Strumigenys maynei* and the large ant *Platythyrea conradti*. We demonstrated that both ants peacefully share the same arboreal nests in spite of the presence of clearly distinct nestmate recognition cues. Because of the large size differences, we hypothesized that each of the two species would benefit from specializing in carrying out particular tasks, in analogy to the size-related division of labor observed in species with size-polymorphic workers. In line with this theory, we found that the tiny ant *S. maynei* was highly efficient at nest defense against intranidal arthropods and alien ant intruders, whereas the large ant *P. conradti* was highly skilled in nest engineering. We argue that the described association is

likely mutualistic, as *P. conradti* clearly benefited from the supreme defense capabilities of *S. maynei*, and that, conversely, *S. maynei* took advantage of small prey thriving in the organic nest material collected by *P. conradti*.

Significance statement

Associations between social insects are typically parasitic in nature. A few cases, however, are known of beneficial associations between social insects. Here, we report such a rare association between two Afrotropical species that share the same nest even though they lack matching colony odors. The large ant *Platythyrea conradti* benefited from the presence of *Strumigenys maynei* as this tiny, but highly aggressive, ant was much more efficient in attacking intranidal and extranidal enemies. *S. maynei* in turn took advantage of *P. conradti* as this ant constructs a unique nest which attracts suitable prey.

Keywords Ant guests · Caste · Nest defense · Polymorphism · Symbiosis · Task specialization

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Introduction

Interspecific mutualisms, i.e. reciprocally beneficial relationships between different species, are ecologically widespread, important at several levels of biological organization and vital for ecosystem functioning (Herre et al. 1999). Mutualisms typically involve organisms that provide food and shelter in return for protection from predators and competitors. Corals, for example, provide shelter and food to herbivorous fish in return for protection from enemies such as seaweeds (Dixon and Hay 2012). Ant nests are safe environments with ample of food sources and are consequently targeted by many organisms (Kronauer and Pierce 2011). Most of these associates do

not return benefits and are clearly parasitic (Kronauer and Pierce 2011; Parmentier et al. 2016). Yet, rare examples of “parabiosis” are known in which the nest is peacefully shared with another ant species (Hölldobler and Wilson 1990; Menzel and Blüthgen 2010). The most widely accepted theory is that parabirotic associations are of a mutualistic nature and are beneficial for both ant partners. For example, in a parabirotic association between two South-East Asian ants, one of the species, *Crematogaster modiglianii*, was shown to benefit from the presence of the stronger and more aggressive *Camponotus rufifemur*, whilst the latter took advantage of the pheromone trails and nest construction capabilities of *Cr. modiglianii* (Menzel and Blüthgen 2010). A similar mutualistic association was also demonstrated in South American parabirotic ants (Davidson 1988; Vantaux et al. 2007). Nevertheless, Menzel et al. (2014) also reported that some parabirotic partners seemed to be exploited, without receiving any return benefits from the partner. Parabirotic ants tolerate the presence of another ant species in the same nest, even if both ant species typically raise their brood in different nest chambers. Surprisingly, such species do not show any aggression towards each other, often exploit the same food sources and may even use the same pheromone trails (Menzel and Blüthgen 2010).

Parabiosis can involve tight associations where both partners show colony-specific tolerance levels. In this case, only the partner colony is tolerated and conspecific and heterospecific workers of other compound nests are aggressed by both partner colonies (Orivel and Dejean 1997; Emery and Tsutsui 2013). Other associations are less strict, as a partner might also tolerate other colonies of the partner (Menzel et al. 2008b). In contrast with most social insect parasites that mimic the odor of their host to get accepted, parabirotic ants succeed to associate even when they each have distinct chemical cuticular profiles (Orivel and Dejean 1997; Menzel et al. 2008a; Menzel et al. 2009). It is suggested that parabirotic ants are able to recognize the chemical profile of the partner using a learning process which leads to colony- or species-specific tolerance (Orivel and Dejean 1997). Nevertheless, there are also indications that a parabirotic lifestyle imposes selection pressures on the chemical profile, as parabirotic ants frequently possess exceptionally long-chain hydrocarbons and higher amounts of methyl-branched alkenes and alkadienes (Menzel and Schmitt 2012) or may carry cuticular compounds that are thought to appease the other partner (Menzel et al. 2013).

In the present study, we explored an apparent parabirotic association between the large Ponerinae ant *Plathythyrea conradti* Emery, 1899 and the tiny Myrmicinae ant *Strumigenys maynei* Forel, 1916 that was recently discovered in the Ivory Coast (Yéo et al. 2006). The aim of our study was threefold. First, we investigated the nature and specificity of the association by measuring the level of aggression

between the two partners and analyzing whether they could discriminate conspecific and heterospecific workers of alien compound nests. Second, we carried out a chemical analysis of the cuticular hydrocarbon profiles of *P. conradti* and *S. maynei* of different compound nests to determine the chemical congruence between the associated ants across different nests. We then linked these chemical data with the behavioral assays and discuss these results with respect to the specificity of the ant association. Finally, we studied the potential benefits for both partners of engaging in the association. In species with size-polymorphic workers, it is well documented that workers of particular size cohorts specialize on carrying out specific tasks inside the colony, such as nest defense (Hölldobler and Wilson 1990; Tian and Zhou 2014; Parmentier et al. 2015). Analogously, we hypothesized that a similar specialization in nest defense between the two ant partners that vary greatly in size and morphology could bring distinct benefits to the association. Defense capabilities were tested for both ant partners towards intranidal intruders (myrmecophiles) and towards extranidal enemies (alien ant species).

Material and methods

Study site and study organisms

P. conradti and *S. maynei* (Fig. 1a) were found in a gallery forest along the Bandama river in the Lamto Ecological Reserve (6° 13' N, 5° 01' W), Ivory Coast, in January 2016 (dry season). The distribution of *S. maynei* (Myrmecinae) is restricted to the forest zones of West and Central Africa and Uganda (Bolton 2000). This tiny (ca. 2.5 mm long) ant is often found nesting not only in rotten wood in the leaf litter layer but also in holes in trees (Bolton 2000). Most species of *Strumigenys* are specialized predators that capture small arthropods (Hölldobler and Wilson 1990; Bolton 1999). Colonies of *S. maynei* produce large number of workers and are headed by multiple queens (polygyne), but in some colonies, only one queen occurs (Yéo et al. 2006, pers. observations TP). *P. conradti* (Ponerinae) is a large (ca. 15 mm long) Afrotropical arboreal ant that produces relatively small colonies (max. 500 workers). The workers hunt solitarily and prey on a wide variety of arthropods that they kill using a powerful sting (Dejean 2011). They have an unusual social system with permanent wingless (“ergatoid”) queens (Molet and Peeters 2006). New *P. conradti* colonies arise by splitting (fission), and a queen with workers subsequently disperses by walking to a new nest site (Molet and Peeters 2006). Both *P. conradti* and *S. maynei* are found in the absence of each other across their overlapping distribution in the Afrotropical region (pers. observations KY, Bolton 2000). However, Yéo et al. (2006) reported that *S. maynei* colonies were present in 9

Fig. 1 Overview of the compound nest microcosm of *Platythyrea conradti* and *Strumigenys maynei* and some interactions. **a** Inhabitants of the compound nest: (1) *P. conradti*, (2) *S. maynei*, (3) *Pselaphinae* sp. (subtribe Batrisina), (4) *Holotrochus* sp. and (5) adults and nymphs of *Neoasterolepisma delamarei*. **b** Aggression between workers of *S. maynei* originating from different nests. **c** Aggression between workers of *P. conradti* originating from different nests. **d** *P. conradti* and *S. maynei* queen, workers and alate queen. **e** Typical compound nest with opening at a height between 1 and 2 m filled with organic material. The dotted line indicates the shape and depth of the nest in the hollow branch. The brown polygon represents the plug of organic material at the nest entrance



of 12 inspected *P. conradti* nests in Lamto. These compound nests were typically found in hollow branches of living trees (usually *Pancovia bijuga*, Sapindaceae) 0.5–2 m above ground. The openings of these branches were stuffed with organic material and faced slightly downwards or upwards (cf Fig. 1e). A number of ant-associated arthropods or myrmecophiles were also detected in the compound nests (pers. observations KY, WD).

In this study, hollow branches of ten living trees, which were characteristically filled with organic material, were opened using a machete. Organic material was collected by hand, whereas ants and myrmecophiles were aspirated. Most organic material was found near the nest entrance (plug with a length of 10–20 cm), but some debris were also found in the deeper cavities of the hollow branch. The organic material was carefully sieved in the lab to find additional ants and

myrmecophiles. Ants and myrmecophiles were housed per nest in plastic 1-L containers with a bottom layer of moist plaster and organic material of the original nest. A cotton plug soaked in sugar water was provided regularly.

The nature and specificity of the association

In a series of aggression experiments, the behavior of *S. maynei* and *P. conradti* towards workers of the partner colony found in the same compound tree nest and towards workers of *S. maynei* and *P. conradti* found in other nests was tested. In all tests, the proportion of aggressive interactions (opening mandibles, biting and stinging) observed during a total of 20 interactions was scored as the dependent variable. We defined an interaction as the crossing of ant antennae with the introduced individual or one of its body parts.

Indeed, the tiny *S. maynei* workers did not interact with the whole body of the much larger *P. conradti* workers but mainly just with their body parts (legs, antennae) that contacted the ground. Test arenas had a plaster bottom and fluon-coated walls to prevent animals from escaping. Because of the large size differences between *S. maynei* and *P. conradti* (Fig. 1a, d), different test arenas and number of workers were used depending on the interaction tested (overview in Table 1)

Aggression tests of *P. conradti* towards *P. conradti* workers of the same colony and alien colonies from other compound nests were done by introducing a *P. conradti* worker into an arena with one *P. conradti* worker. Both workers originated from nest N₁, N₂ or N₃, but tests were done blind to the origin of the introduced worker. Both workers were replaced in every trial.

Aggression of *P. conradti* towards co-inhabiting and alien workers of *S. maynei* was tested by introducing one *S. maynei* worker into an arena (diameter 8 cm) with 10 workers of *P. conradti*. Workers originated from nest N₁ and N₂, but tests were again done blind with respect to the origin of the *S. maynei* worker.

Aggression of *S. maynei* towards nestmate and alien *P. conradti* workers was analyzed in an arena with a diameter of 3 cm. Here, the behavior of three individuals of *S. maynei* towards one *P. conradti* worker was followed. These tests were done with two colonies of *S. maynei* from nests N₁ and N₃, and workers were replaced in every trial. *P. conradti* workers also originated from nests N₁ and N₃, but tests were performed blind with respect to the origin of the *P. conradti* workers. Interactions were scored after the *P. conradti* worker calmed down and no longer walked around, whereas in all other tests described in the following section, aggression

scoring was recorded starting 10 s after introduction of an intruder into the arena.

Aggression of *S. maynei* towards workers of alien *S. maynei* colonies was tested by introducing a *S. maynei* individual in an arena (diameter 8 cm) with 40 *S. maynei* workers from nest N₁, N₂ or N₃. The introduced *S. maynei* individuals belonged to one of these colonies, but tests were performed blind with respect to the origin of these workers. Aggression tests with *S. maynei* as defender were observed under a Leica MZ6 stereo-microscope.

The effect of nest origin, i.e. same nest or alien nest, of an introduced *S. maynei* worker on the proportion of aggressive interactions elicited in an arena with 40 *S. maynei* workers were analyzed using a generalized linear mixed model (GLMM) with a binomial error distribution using R package lme4. Significance was tested using a likelihood ratio test using R package car. The nest origin of the introduced worker was included as a fixed factor, whereas the nine possible combinations of host and introducer colony (nest of host colony-nest of introducer colony: N₁-N₁, N₁-N₂, N₁-N₃, N₂-N₁, N₂-N₂, N₂-N₃, N₃-N₁, N₃-N₂ and N₃-N₃) were included as a random intercept. In addition, an observation-level random intercept was incorporated to account for possible overdispersion (Browne et al. 2005). A similar model was run to assess the effect of nest origin of an introduced *P. conradti* worker on the proportion of aggressive interactions elicited in an arena with three *S. maynei* workers. As the two ant species originated from only two nests, the random factor that implemented the combination of acceptor and introducer colony had only four levels (nest of host colony-nest of introducer colony: N₁-N₁, N₁-N₃, N₃-N₁ and N₃-N₃). Aggression of *P. conradti* towards other *P. conradti* workers and towards *S. maynei*, either from

Table 1 Overview of the aggression experiments

Intruder	Nest origin intruder	Host	Host workers in arena (N)	Arena diameter
Ants				
<i>P. conradti</i>	Same nest	<i>P. conradti</i>	1	8 cm
<i>P. conradti</i>	Alien nest	<i>P. conradti</i>	1	8 cm
<i>S. maynei</i>	Same nest	<i>P. conradti</i>	10	8 cm
<i>S. maynei</i>	Alien nest	<i>P. conradti</i>	10	8 cm
<i>P. conradti</i>	Same nest	<i>S. maynei</i>	3	3 cm
<i>P. conradti</i>	Alien nest	<i>S. maynei</i>	3	3 cm
<i>S. maynei</i>	Same nest	<i>S. maynei</i>	40	8 cm
<i>S. maynei</i>	Alien nest	<i>S. maynei</i>	40	8 cm
Alien ants				
5 species	Extranidal	<i>P. conradti</i>	10	8 cm
5 species	Extranidal	<i>S. maynei</i>	40	8 cm
Myrmecophiles				
6 species	Same nest	<i>P. conradti</i>	10	8 cm
6 species	Same nest	<i>S. maynei</i>	40	8 cm

the same or an alien nest, was not modelled as no variation was observed within a treatment.

The number of trials for each interaction is listed in Table 2.

Cuticular hydrocarbon profiles of the ant partners

Cuticular compounds of freeze-killed *S. maynei* workers (five samples from nest N₄, three samples from nest N₅) were extracted in 30 µL of hexane (HPLC, Sigma-Aldrich) in 2-mL glass vials with PTFE septum (Sigma-Aldrich) for 10 min. Because of their small size, five *S. maynei* workers were pooled per sample. The large *P. conradti* workers (five samples from each of nests N₄, N₅ and N₆) were extracted in 200 µL of hexane for 10 min. The samples of the *S. maynei* colony from N₆ were contaminated, and only the *P. conradti* colony of that nest was therefore analyzed. Samples were evaporated at room temperature to dryness and stored at −18 °C. Prior to analysis, *S. maynei* samples were diluted again in 30 µL hexane and *P. conradti* samples in 200 µL hexane, and 2 µL of these solutions were injected into a GC/MS (Thermo Fisher: TRACE 1300 gas chromatograph, ISQ series mass spectrometer) equipped with a Restek Rxi-5Sil MS column (20 m × 0.18 mm × 0.18 µm). The method had an initial temperature profile consisting of 1 min at 40 °C, two

temperature ramps from 40 to 200 °C at 20 °C min^{−1} and from 200 to 340 °C at 8 °C min^{−1}, after which the final temperature of 340 °C was held for 4 min. We used helium as a carrier gas at a flow rate of 0.9 mL min^{−1}, splitless injection and an inlet temperature of 290 °C. All samples and a linear C7 to C40 linear alkane ladder standard (49452-U, Supelco) at a concentration of 0.001 and 0.01 µg/mL were run in the same batch. Retention indices were calculated using cubic spline interpolation based on the elution times of the external alkane ladder standard. These calculations were done using an in-house developed R script (available from the authors on request).

For both ant species, we calculated the mean relative percentage of every compound and selected the compounds that had a higher relative percentage than 0.1% on average. These compounds were present in all individual samples of either *S. maynei* or *P. conradti*, even though some compounds composed less than 0.1% of the individual cuticular profile. Peaks were identified on the basis of their retention index and mass spectra. The analysis of the level of similarity among cuticular profiles was based on the hydrocarbon peaks that were shared by both ants to avoid the effect of shared absences. It is generally assumed that hydrocarbons are pivotal in nestmate recognition in ants (van Zweden and D'Ettorre 2010). The areas of the hydrocarbon peaks were first transformed by the

Table 2 Mean proportion of aggressive interactions of *S. maynei* and *P. conradti* towards inhabitants of the same or alien nests

Introduced species		<i>N</i>	<i>Platythyrea conradti</i>	<i>N</i>	<i>Strumigenys maynei</i>
Ants					
<i>Platythyrea conradti</i> same nest		20	0.00	30	0.07 [0.05–0.10]
<i>Platythyrea conradti</i> alien nest		15	1.00	30	0.05 [0.03–0.08]
<i>Strumigenys maynei</i> same nest		20	0.00	18	0.02 [0.01–0.05]
<i>Strumigenys maynei</i> alien nest		20	0.00	36	0.42 [0.26–0.59]
Alien ants					
<i>Monomorium pharaonis</i>		10	0.00	5	0.91 [0.82–0.97]
<i>Monomorium bicolor</i>		10	0.03 [0.01–0.06]	5	0.91 [0.82–0.97]
<i>Crematogaster</i> sp. 1		10	0.01 [0.00–0.03]	4	0.90 [0.79–0.96]
<i>Crematogaster</i> sp. 2		10	0.02 [0.00–0.04]	5	0.98 [0.92–1.00]
<i>Oecophylla longinoda</i>		10	0.08 [0.04–0.13]	5	0.90 [0.81–0.96]
Myrmecophiles					
Coleoptera					
<i>Pselaphinae</i> sp. 1	Subtribe Batrisina	9	0.01 [0.00–0.05]	7	0.58 [0.49–0.66]
<i>Scydmaeninae</i> sp.	“ <i>Napocornus</i> complex” of genera	5	0.00	5	0.42 [0.32–0.52]
<i>Holotrochus</i> sp.	Staphylinidae: Osoriinae	8	0.00	5	0.56 [0.46–0.66]
Collembola					
<i>Cyphoderus subsimilis</i>	Cyphoderidae	5	0.00	5	0.45 [0.35–0.55]
Thysanura					
<i>Neoasterolepisma delamarei</i>	Lepismatidae	9	0.25 [0.15–0.37]	4	0.83 [0.73–0.90]
<i>Mesonychographis myrmecophila</i>	Nicoletiidae: Atelurinae	3	0.00	–	–

Ninety-five percent confidence intervals in brackets

N number of trials

Aitchison's log-ratio transformation (Aitchison 1986), and samples were then grouped by a hierarchical cluster analysis (Euclidean distances, Ward's method) using the R function *hclust*. Finally, we examined which compounds were likely to be environmentally (i.e. shared by the two parabiotic ant species within a nest but not across nests) or genetically (i.e. shared by the same species across nests but not between species) determined. Therefore, we conducted a permutational multivariate analysis of variance using distance matrices (*adonis* function of the R package *vegan*, 1000 permutations). The factors "species" and "nest" and their interaction were modelled as explaining factors and a distance matrix with the Euclidean distances among the chemical profiles (Aitchison's transformed hydrocarbon dataset) of the parabiotic ants as response. As we did not have cuticular hydrocarbon (CHC) samples of *S. maynei* collected in N₆, we dropped the five *P. conradti* samples of this nest from the dataset to have a fully crossed design (*P. conradti* and *S. maynei* samples from N₄ and N₅).

Potential benefits of the association

A diverse group of myrmecophiles was collected in the 10 inspected nests (Online Resource, Table S1). It is unknown whether they are strictly associated with ants ("obligate myrmecophiles") or can be found in absence of ants as well ("facultative myrmecophiles"). However, three species, i.e. the springtail *Cyphoderus subsimilis* and the silverfish *Neoasterolepisma delamarei* and *Mesonychographis myrmecophila*, are expected to be obligate myrmecophiles, based on previous records with ants and/or a strict myrmecophile relationship of related taxa (Table S1). Many myrmecophiles impose costs on their host by preying on their brood or by feeding on prey brought to the nest (Kronauer and Pierce 2011; Parmentier et al. 2016). Similarly, most myrmecophiles in the parabiotic nest are expected to impose costs on the parabiotic hosts. Therefore, an efficient defense strategy against these intruders could be beneficial for both parabiotic ant partners. Here, we tested whether one ant partner was more efficient in detecting and attacking these intruders. Aggression of *P. conradti* and *S. maynei* was tested towards six myrmecophiles that were common in the nests (Table S1): *Pselaphinae* sp. 1 (Coleoptera), *Scydmaeninae* sp. (Coleoptera), *Holotrochus* sp. (Coleoptera, Staphylinidae), *Cyphoderus subsimilis* (Collembola), *Neoasterolepisma delamarei* (Thysanura) and *Mesonychographis myrmecophila* (Thysanura). Except for the springtail *Cyphoderus subsimilis*, they are expected to impose costs on their host. In addition to intranidal enemies, ant nests can be attacked by extranidal enemies. The most important enemies of ants are other ants (Hölldobler and Wilson 1990). Therefore, aggression of both parabiotic ants was also tested towards five ant species, which were very common in the study area. Ant species were selected along a size gradient (from small to large: *Monomorium*

pharaonis, *Monomorium bicolor*, *Crematogaster* sp. 1, *Crematogaster* sp. 2, *Oecophylla longinoda*) to assess whether the relative size of the ant enemy affected aggression of *P. conradti* or *S. maynei*. Aggression tests of *P. conradti* and *S. maynei* towards myrmecophiles and alien ants (Table 1) were conducted similarly as described in the previous aggression trials. Myrmecophiles were introduced in an arena (diameter 8 cm) with 10 workers of *P. conradti* found in the same nest (nests N₂, N₃, N₆, N₇ and N₈); workers of alien ant species were introduced in an arena (diameter 8 cm) with 10 *P. conradti* workers of nest N₅. Aggression of *S. maynei* towards myrmecophiles and alien ant workers was tested in the same way, but observations were done under a Leica MZ6 stereo-microscope. Myrmecophiles here were introduced in test arenas with 40 *S. maynei* workers collected in the same nest (nests N₃, N₅ and N₆); alien ants were introduced in an arena with 40 *S. maynei* workers of nest N₅. Different myrmecophile and alien ant individuals were used per trial. To assess the confidence intervals listed under alien ants and myrmecophiles in Table 2, we ran four different quasibinomial generalized linear models. In particular, the proportion of aggression of *S. maynei* towards alien ants (model 1) and myrmecophiles (model 2) and the proportion of aggression of *P. conradti* towards alien ants (model 3) and towards myrmecophiles (model 4) were modelled as response variable and species as explaining factor.

Results

The nature and specificity of the association

Colonies of *P. conradti* and *S. maynei* were always found together in the 10 inspected branches. Brood of both species was present in most of the inspected nests but was clearly separated. The ten colonies of *S. maynei* were all polygynous and contained multiple breeding queens, and winged male and female sexuals were also recorded (Fig. 1d). In one *P. conradti* colony, male sexuals were observed. *P. conradti* did not show any aggression towards workers of *S. maynei* living in the same compound nest or coming from an alien nest (Table 2, Online Resource video S1). In contrast, alien *P. conradti* workers were directly and fiercely attacked by biting and stinging (Fig. 1c). The workers involved in the fight could not be separated and fought until death. Therefore, the proportion of aggressive interactions in Table 2 was set to one. Like *P. conradti*, *S. maynei* was very aggressive towards conspecific workers of an alien nest (GLMM, likelihood ratio test, $df = 1$, $\chi^2 = 32.56$, $P < 0.001$) (Fig. 1b).

S. maynei showed limited aggression towards *P. conradti* living in the same nest, and aggression was not elevated when *P. conradti* originated from an alien nest (GLMM, likelihood ratio test, $df = 1$, $\chi^2 = 1.33$, $P = 0.248$) (Table 2, Online Resource S1). A power analysis for this mixed model was

performed using the R package *simr*. It showed that an increase in the proportion of aggressive interactions from 0.07 (aggression of *S. maynei* against *P. conradti* nestmates) to 0.17, 0.27, 0.37 and 0.47 could be detected with a power of, respectively, 17.1, 42.9, 69.8 and 90.0%. This implies that a small increase in aggression is unlikely to be detected with our setup. However, we showed that *S. maynei* strongly elevates its aggression towards alien *S. maynei* workers compared to workers of its own colony. When *S. maynei* would be able to recognize its *P. conradti* nest partner, a similar large increase in aggression could be expected towards alien *P. conradti*. This expectation is in line with the findings of a previous study (Orivel and Dejean 1997). Here, it was shown that parabiotic ants, which are able to recognize the partner colony, dramatically increased aggression towards alien partners.

S. maynei was never observed soliciting for food, and grooming behavior between heterospecific workers was also absent. No interspecific brood predation was observed between the ant partners in lab nests.

Cuticular hydrocarbon profiles of the ant partners

A total of 78 different peaks were distinguished across both ant species. The majority of the peaks consisted of linear hydrocarbons ($N = 59$) (Table 3), which could be identified based on their mass spectra and retention indices. There were also non-hydrocarbon compounds which we did not identify ($N = 19$). The profile of *P. conradti* was distinct from that of *S. maynei*, with a much higher proportion of light hydrocarbons (between n-C21 and n-C27) (Table 3, Fig. 3). Nevertheless, both species shared 30 hydrocarbon peaks (Table 3, Fig. 3). The summed concentrations of these 30 hydrocarbons contributed to 91% of the total CHC concentration in *S. maynei* and 89% in *P. conradti* and represent, thus, the majority of the nestmate recognition cues. *P. conradti* and *S. maynei* formed two distinct clusters in the hierarchical cluster analysis and the workers grouped per nest within both clusters (Fig. 4). This was confirmed by the multivariate permutation test which showed a very strong (explained 78.8% of the total CHC variation across samples) species effect on hydrocarbon composition ($F = 110.50$, $P < 0.001$). There were also small effects of the nest environment (explained 5.8% of the total CHC variation across samples) ($F = 8.19$, $P = 0.02$) and the interaction effect (explained 5.4% of the total variation, $F = 7.53$, $P = 0.01$) on an individual's CHC composition. However, these results are based on merely two nests, and a larger dataset should be needed to assess more precisely how the common nest environment affects the hydrocarbon profile of the parabiotic ants.

Potential benefits of the association

Myrmecophiles and alien ants elicited no or only limited aggression in *P. conradti* (Table 2). Surprisingly, *P. conradti*

avoided alien ants and regularly tried to escape even when the introduced workers were much smaller (Online Resource videos 2, 3). This can be seen for the total number of escapes out of total number of interactions in trials with: *Monomorium bicolor* $N = 8/200$, *Crematogaster* sp. 1 $N = 8/200$, *Crematogaster* sp. 2 $N = 20/200$ and *O. longinoda* $N = 8/200$. *P. conradti* seldom initiated a fight with an alien ant and showed in general merely aggression when it was bitten or stung by the introduced alien ant worker. Alien ants and myrmecophiles evoked a strong aggression response in *S. maynei*, in clear contrast to what we observed in *P. conradti*. *S. maynei* workers typically grabbed the legs of the intruder and tried to sting (Online Resource: videos 4, 5, 6, 7 and 8). An overview of the tested interactions between the inhabitants of the compound nests is schematically illustrated in Fig. 2.

We observed *S. maynei* with small prey collected from the organic nest material (three times with Collembola: *Cyphoderus subsimilis*, twice with Pseudoscorpiones) held between their mandibles in lab nests.

Discussion

In this study, we identified a unique association between two Afrotropical ants. Colonies of the tiny ant *Strumigenys maynei* and the large ant *Platythyrea conradti* lived together in all inspected tree nests in the study area. There was little or no aggression between the two partners, but there were also no signs of intimate heterospecific interactions such as trophallaxis or grooming which are observed between ants and specialized myrmecophiles or social parasites (Hölldobler and Wilson 1990; Buschinger 2009). Because of the strict association and the lack of aggression between the two partners, this association can be considered as a parabiosis.

Our results confirmed that parabiotic partners might associate with each other despite having distinct nestmate recognition cues (Orivel and Dejean 1997; Menzel et al. 2008a; Menzel et al. 2009; Emery and Tsutsui 2013) (Figs. 3 and 4). Previous studies showed that parabiotic partners hardly shared any cuticular compounds (summarized in Table 1 in Emery and Tsutsui 2013). *P. conradti* and *S. maynei*, in contrast, had 51% of hydrocarbons in common. CHC composition was slightly affected by the nest environment (e.g. by passive transfer between the parabiotic partners, nest material, diet...), but the cuticular cues of an individual were mainly determined by species identity ("genetic"). It is unclear, however, whether the parabiotic lifestyle of the ants of this study exerts selection on the presence and proportional composition of nestmate recognition cues (cf. Menzel et al. 2013) to facilitate the recognition and/or acceptance in the association. The ants clearly perceive conspecific workers with a slightly different chemical profile in both species as they showed strong aggression against conspecific workers. Tolerance of the

Table 3 Comparison of cuticular components (average percentages \pm SD) of *S. maynei* ($N_{\text{pooled}} = 9$) and *P. conradti* ($N = 15$)

Peak no.	Retention index		<i>S. maynei</i>	<i>P. conradti</i>	Peak no.	Retention index		<i>S. maynei</i>	<i>P. conradti</i>
1	2026.78	Non-HC	0.33 \pm 0.13	—	40	2686.00	Non-HC	0.27 \pm 0.54	—
2	2053.23	Non-HC	0.50 \pm 0.54	—	41	2686.73	4,16-diMeC26	—	1.41 \pm 0.71
3	2100.02	n-C21	0.22 \pm 0.06	0.32 \pm 0.25	42	2699.59	n-C27	7.37 \pm 2.63	4.56 \pm 1.98
4	2130.00	Non-HC	0.28 \pm 0.33	—	43	2732.58	13,11,9-MeC27	3.00 \pm 1.96	8.45 \pm 2.48
5	2149.00	Non-HC	8.56 \pm 13.68	—	44	2750.09	5-MeC27	0.21 \pm 0.11	0.52 \pm 0.15
6	2172.96	3-MeC21	—	0.67 \pm 0.71	45	2760.52	Non-HC	6.51 \pm 14.07	0.41 \pm 0.44
7	2199.84	n-C22	0.22 \pm 0.11	0.18 \pm 0.12	46	2773.96	3-MeC27	8.74 \pm 2.90	4.98 \pm 1.57
8	2276.74	C23:1	—	0.25 \pm 0.27	47	2781.75	5,y-diMeC27	0.66 \pm 0.19	0.77 \pm 0.22
9	2299.58	n-C23	0.34 \pm 0.17	2.94 \pm 1.16	48	2799.17	n-C28	1.15 \pm 0.36	0.13 \pm 0.09
10	2303.00	Non-HC	0.48 \pm 0.34	—	49	2806.40	3,y-diMeC27	2.23 \pm 1.42	1.90 \pm 0.54
11	2335.71	11,9-MeC23	—	1.68 \pm 0.50	50	2831.48	12-MeC28	—	0.38 \pm 0.08
12	2341.78	7-MeC23	—	0.31 \pm 0.22	51	2832.00	Non-HC	0.68 \pm 0.21	—
13	2350.62	5-MeC23	—	0.45 \pm 0.17	52	2839.97	Non-HC	0.58 \pm 0.42	0.45 \pm 0.25
14	2373.01	3-MeC23	—	2.33 \pm 1.58	53	2850.61	Non-HC	10.88 \pm 10.60	0.82 \pm 0.39
15	2377.00	Non-HC	0.14 \pm 0.09	—	54	2862.00	4-MeC28	1.58 \pm 0.46	—
16	2398.00	Non-HC	0.56 \pm 0.36	—	55	2869.00	Non-HC	1.13 \pm 0.62	—
17	2399.56	n-C24	—	0.56 \pm 0.27	56	2869.84	x-C29:1	—	0.64 \pm 0.31
18	2408.97	3,13-diMeC23	—	0.71 \pm 1.24	57	2879.34	y-C29:1	0.74 \pm 0.47	1.65 \pm 0.81
19	2415.00	Non-HC	0.14 \pm 0.06	—	58	2888.87	Non-HC	—	0.15 \pm 0.06
20	2434.72	x-MeC24	0.17 \pm 0.03	1.11 \pm 0.30	59	2899.04	n-C29	4.73 \pm 1.80	0.27 \pm 0.14
21	2445.26	6-MeC24	—	0.25 \pm 0.13	60	2930.37	15,13,11,9-MeC29	2.19 \pm 0.72	0.93 \pm 0.28
22	2458.02	4-MeC24	—	0.21 \pm 0.17	61	2940.00	7-MeC29	0.25 \pm 0.20	—
23	2477.21	x-C25:1	—	1.88 \pm 0.77	62	2950.00	5-MeC29	0.40 \pm 0.22	—
24	2484.73	y-C25:1	—	0.46 \pm 0.21	63	2962.05	x,y-diMeC29	1.31 \pm 0.55	0.13 \pm 0.05
25	2486.00	Non-HC	0.98 \pm 0.67	—	64	2973.11	3-MeC29	4.28 \pm 1.14	0.19 \pm 0.05
26	2492.54	4,14-diMeC24	—	0.21 \pm 0.13	65	2980.09	5,y-diMeC29	—	0.10 \pm 0.03
27	2499.80	n-C25	1.13 \pm 0.55	5.36 \pm 2.64	66	3000.00	n-C30	0.17 \pm 0.07	—
28	2534.29	13,11,9-MeC25	0.68 \pm 0.88	10.74 \pm 1.52	67	3030.00	x-MeC30	0.67 \pm 0.43	—
29	2541.89	7-MeC25	0.30 \pm 0.45	2.01 \pm 1.32	68	3052.34	Non-HC	—	0.14 \pm 0.09
30	2550.63	5-MeC25	—	1.04 \pm 0.44	69	3056.00	Non-HC	1.07 \pm 0.45	—
31	2573.83	3-MeC25	1.58 \pm 0.82	7.58 \pm 2.10	70	3069.92	C31:1	0.18 \pm 0.09	0.31 \pm 0.21
32	2582.63	5,y-diMeC25	0.27 \pm 0.23	1.56 \pm 0.64	71	3080.70	C31:1	0.15 \pm 0.09	0.23 \pm 0.19
33	2599.71	n-C26	0.45 \pm 0.25	1.19 \pm 0.49	72	3100.00	n-C31	0.94 \pm 0.77	—
34	2607.99	3,y-diMeC25	0.72 \pm 0.46	3.42 \pm 1.91	73	3129.40	15,13,11,9-MeC31	2.27 \pm 1.54	0.62 \pm 0.29
35	2633.19	x-MeC26	0.23 \pm 0.22	2.64 \pm 0.40	74	3178.38	Non-HC	10.77 \pm 5.32	1.94 \pm 1.49
36	2644.41	6-MeC26	—	0.25 \pm 0.09	75	3228.00	Non-HC	1.11 \pm 0.70	—
37	2657.98	4-MeC26	—	0.44 \pm 0.21	76	3300.00	n-C33	0.21 \pm 0.09	—
38	2662.00	Unknown HC	0.64 \pm 0.42	—	77	3328.47	x-MeC33	1.34 \pm 0.81	0.22 \pm 0.10
39	2678.57	C27:1	2.14 \pm 5.07	16.81 \pm 4.49	78	3527.26	x-MeC35	1.17 \pm 0.44	0.13 \pm 0.08

HC hydrocarbon, Non-HC non-hydrocarbon component

parabiotic species can be limited to a single heterospecific partner colony. In this case, there is no aggression between parabiotic partners of the same nest, but both species are aggressive towards allocolonial (=from another compound nest) workers of their partner species (Orivel and Dejean 1997; Emery and Tsutsui 2013). It is suggested that the partners

learn to recognize the distinct chemical odor of their partner colony (Orivel and Dejean 1997). Other associations are less specific and are characterized by complete or a gradient of tolerance towards allocolonial workers from the partner species (Menzel et al. 2008b). The association between *S. maynei* and *P. conradti* is also not specific, as there is no elevated

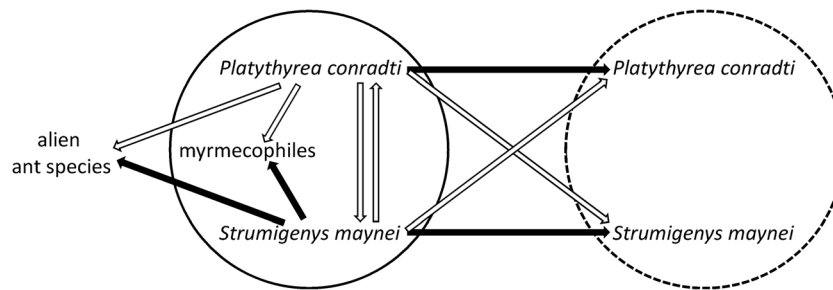


Fig. 2 Schematic overview of aggressive interactions in *Platythreya conradti*-*Strumigenys maynei* compound nests based on Table 2. The solid circle symbolizes the focal nest and the dashed circle an alien nest. Arrows refer to an interaction between *P. conradti* or *S. maynei*

colonies towards species at the end of the arrow. White arrows indicate no or minimal aggression, whereas black arrows designate overt aggression

aggression towards allocolonial workers of the partner. Both species apparently accept all colonies from the partner species. This can be explained by the recognition of species-specific rather than colony-specific chemical cues or the detection of appeasing cues (Menzel et al. 2013). However, the tolerance of the parabiotic partner might also be caused by a merely mechanistic process. Possibly *S. maynei* is too small to be detected efficiently by *P. conradti*. However, it was reported that *P. conradti* detected *S. maynei* when it fed on its prey and carried them away (Yéo et al. 2006). On the other hand, *P. conradti* workers might be too large to be attacked by *S. maynei*

workers. It should be noted here that they successfully attacked *Paltothyreus tarsatus*, an ant which equals the size of *P. conradti* (pers. observations TP).

Yéo et al. (2006) found two dealate inseminated queens of *S. maynei* in a small *P. conradti* colony. This suggests that *P. conradti* is the founder of the compound nest and that a *S. maynei* colony do not fission when its partner *P. conradti* colony splits. It appears rather that *S. maynei* queens track *P. conradti* soon after migration to a new nest site. Multiple *S. maynei* queens might found a new colony (primary polygyny) in the *P. conradti* nest, or new queens might be accepted in a well-established *S. maynei* colony (secondary polygyny).

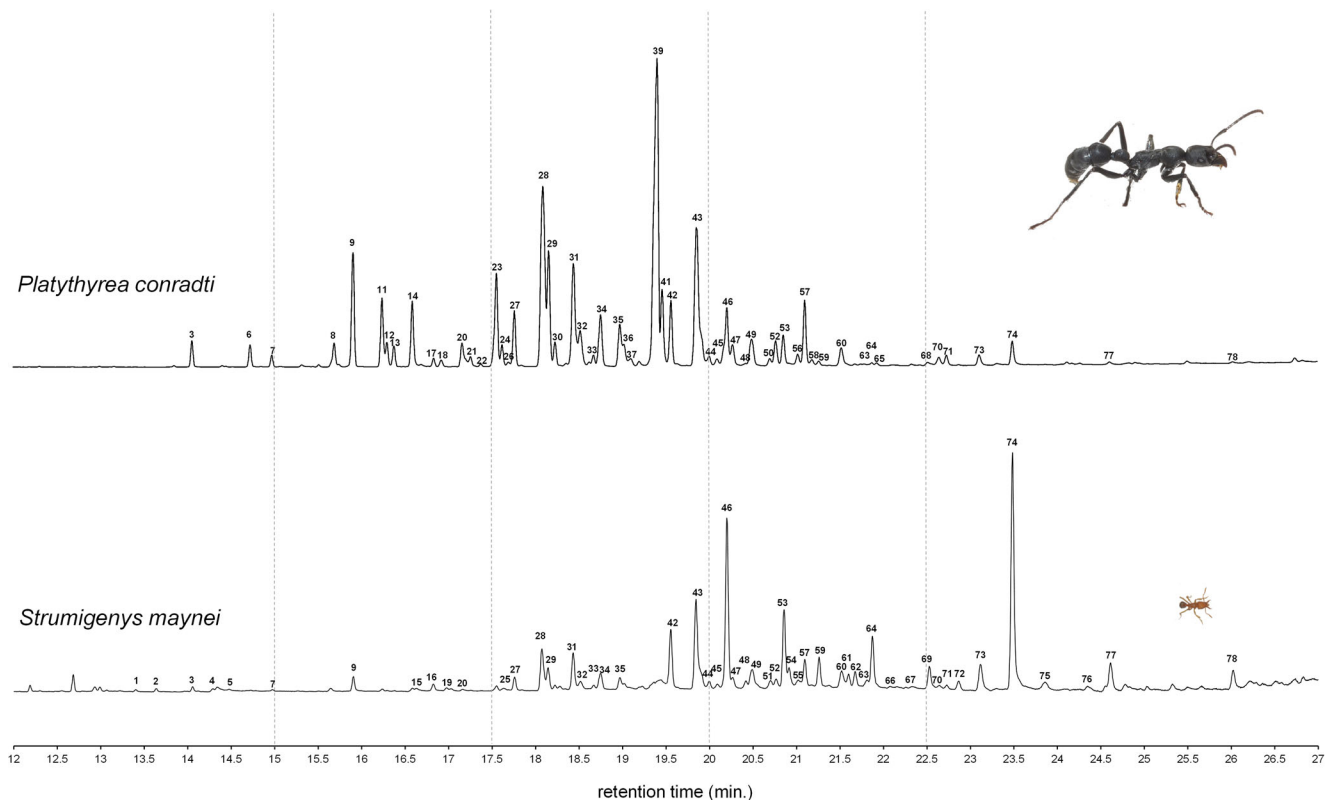


Fig. 3 Representative gas chromatograms of the two co-inhabiting ant species with the relative intensity of peaks in function of retention time. The identity of the peaks corresponding with the peak numbers is given in Table 3

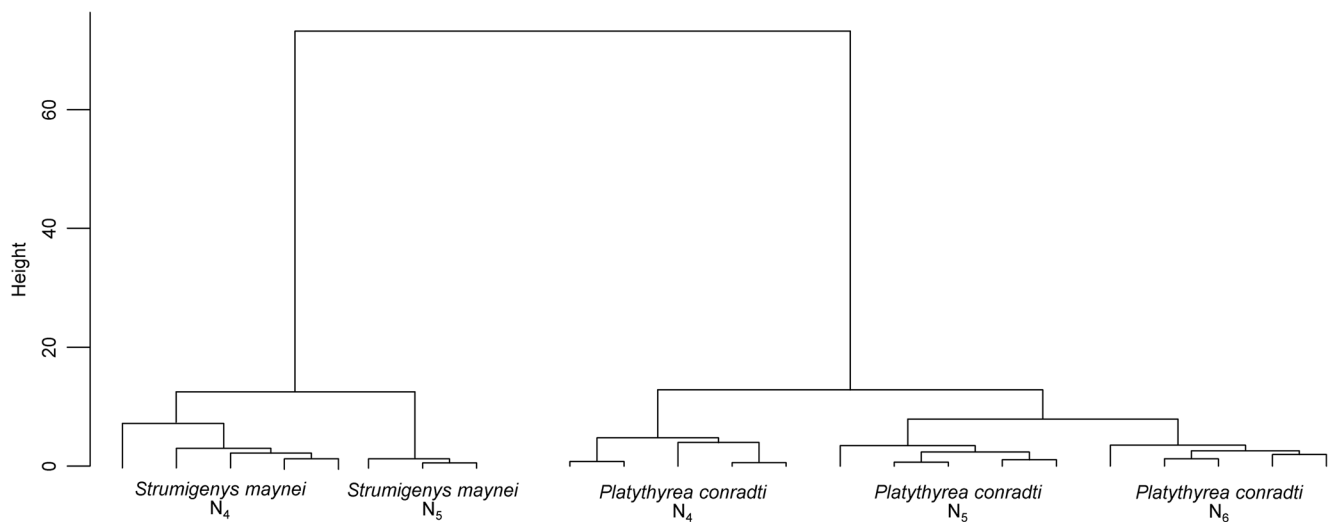


Fig. 4 Hierarchical cluster analysis of the shared cuticular hydrocarbons of *Platythyrea conradti* and *Strumigenys maynei* (Euclidean distance, Ward's method). Colonies of *S. maynei* and *P. conradti* were analyzed

from compound nests N₄ and N₅, the samples of the *S. maynei* colony from N₆ were contaminated and only the *P. conradti* colony of that nest was therefore analyzed

Both ant species seem to benefit from the presence of the other partner. We argue that *P. conradti* takes advantage of *S. maynei*, because the latter is much more efficient in nest defending. Typical threats for ant nests are competitor ants that may rob and destroy the colony and associated myrmecophiles that can prey on the brood and steal prey (Hölldobler and Wilson 1990; Parmentier et al. 2016). Remarkably, the large *P. conradti* workers ignored all myrmecophiles, except for *N. delamarei*. Probably, the relative large size of the latter compared with the other myrmecophiles renders them more detectable for *P. conradti*. *P. conradti* also ignored competitor ants, irrespective of their size, or even tried to escape. Fights were never initiated by this ant, and aggressive behavior was only observed after it was attacked. In a previous study, it was described that these ants crouched with their mandibles open and folded their antennae backwards when they were confronted with competitor ants at a feeding site (Dejean 2011). It was hypothesized that *P. conradti* opened its mandibles to release repellent volatiles secreted by the mandibular glands (Dejean 2011). This peculiar crouching behavior was also observed in our behavioral trials but rarely in combination with mandible opening. In clear contrast, *S. maynei* displayed overt aggression towards myrmecophiles and towards alien ants, irrespective of their size. They typically clung to the legs of the enemies and folded their abdomen to sting. It can be expected that the rather passive *P. conradti* colonies highly benefit from the presence of a large legion of very aggressive *S. maynei* workers. The presented association is peculiar because of the large size differences between the two partners. The large worker force of tiny *S. maynei* ants is particularly efficient to repel small intruders and competitors, which are largely overlooked by the large *P. conradti* workers. By analogy, small workers in polymorphic red wood ants were demonstrated to be supreme defenders against

small, intranidal myrmecophilous parasites (Parmentier et al. 2015). The large *P. conradti* workers might be more suited to repel large arthropods or vertebrates in parallel with the defense specialization of large workers in polymorphic ant colonies against large enemies (Lamon and Topoff 1981; Hölldobler and Wilson 1990; Batchelor et al. 2012). There is a vast amount of literature that stresses the specialization of morphologically distinct worker castes in nest defense (Jandt et al. 2013; Tian and Zhou 2014), but here, we argue that morphologically distinct ant species can be analogously specialized in different tasks. These distinct morphs in the compound nest could be an alternative strategy for worker polymorphism in a single colony of an ant species. Worker polymorphism is assumed to benefit colony fitness as some worker castes are more efficient in the performance of certain tasks (Oster and Wilson 1978; Billick and Carter 2007; Modlmeier and Foitzik 2011; Jandt et al. 2013). Rather than diversifying the morphology of their own worker caste, ants might form a mutualistic association with a morphologically distinct ant species which is more efficient in certain tasks.

S. maynei colonies, in their turn, might also benefit from the parabioc association. *P. conradti* workers fill the nest entrances with a plug of fine and coarse organic material (Fig. 1e) and as such create a microcosm for small arthropods. This can be demonstrated by the enormous abundance of mainly Collembola (*Cyphoderus subsimilis*) that were regularly detected inside the nests (Online Resource Video 9). The genus *Strumigenys* is a group of small predators that capture living prey with their odd-shaped mandibles (Bolton 1999). In this study and in Yéo et al.'s (2006), it was demonstrated that *S. maynei* captured intranidal prey. It appears that *S. maynei* indirectly profits from the nest engineering skills of *P. conradti* to feed on prey living inside the compound nest. Previously, it was observed that *P. conradti* hunted actively

several arthropods in the tree canopy in the rainy season, whereas *S. maynei* workers never foraged further than 10 cm away from the nest entrances (Yéo et al. 2006). This further suggests that *S. maynei* finds its food inside the nest.

Given the apparent benefits for both partners in this parabiatic association and the absence of potential costs, i.e. no food competition and brood predation, this parabiosis is expected to be mutualistic in nature. This is in line with previous studies on parabioses in the Neotropical and Oriental associations between *Camponotus* and *Crematogaster* species which gave evidence that the association was favorable for both parabiatic partners. *Crematogaster* takes advantage of *Camponotus*'s ability to construct ant garden nests and its supreme nest defending abilities (Davidson 1988; Vantaux et al. 2007; Menzel and Blüthgen 2010). *Camponotus* benefits from *Crematogaster* through following its pheromone trails to food sites (Vantaux et al. 2007; Menzel and Blüthgen 2010). However, parabioses between *Camponotus* and *Crematogaster* can also shift to commensalism and parasitism, when there is aggressive competition, exploitation and no apparent benefits for one partner (Menzel et al. 2014).

The parabiatic system of this study is an excellent model system to test interactions between symbiotic arthropods. Further behavioral, ecological and chemical studies that compare the strategies of *S. maynei* and *P. conradti* living in association compared to free-living colonies of both species could greatly contribute to our knowledge on the factors that promote the association and cooperation of two distinct species. Moreover, this study raises an interesting hypothesis that this proposed defense mutualism between size-polymorphic ant species is an adaptive alternative to the evolution of a defensive worker polymorphism within one species.

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